REMARKS

Applicants acknowledge the finality of the restriction requirement. In response to the Examiner's statement that the Matsumoto *et al.* reference was not considered because a copy of the reference was not received, Applicants hereby submit a copy of the Matsumoto *et al.* (Ortho. Res. Soc. Trans.) reference cited in the previously submitted PTO-1449.

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow. Claims 9-13 and 20 are being withdrawn as being drawn to a non-elected invention. Claim 14 has been cancelled. Claims 1, 2, 4, 15, 16 and 18 are currently being amended. Claims 1 and 15 have been substantively amended, whereas claims 2, 4, 16, and 18 have been amended to conform with the amendments to claim 1 and claim 15. The amendment to the claims does not add new matter and is supported throughout the specification and claims as originally filed. For example, support for the amendment of claim 1, can be found in paragraph [0034] and Example 2 of the specification. After amending the claims as set forth above, claims 1-20 are now pending in this application.

This amendment adds, changes and/or deletes claims in this application. A detailed listing of all claims that are, or were, in the application, irrespective of whether the claims remain under examination in the application, is presented, with an appropriate defined status identifier.

I. Claim Rejections

a. 35 U.S.C. § 112

In the Office Action, the Examiner rejected claims 1-8 and 14-19 under 35 U.S.C. § 112, second paragraph for being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 14 has been cancelled, rendering the rejection of this claim moot.

Concerning line 5 of claim 1 and bridging lines 5 and 6 of claim 15, the Examiner stated that "cell-associated on" is uncertain as to meaning. The Examiner suggested that the phrase "matrix" should be inserted in these spots after the term "cell-associated." Applicants

have followed the Examiner's suggestions and amended claim 1 and claim 15 so that the phrase "matrix" follows the phrase "cell-associated" in amended claim 1 and amended claim 15. Because Applicants believe that the claims as amended satisfy 35 U.S.C. § 112, second paragraph, Applicants respectfully request that the Examiner withdraw the rejection.

The Office Action further stated that step (e) of claim 2 and step (v) of claim 16 were unclear because the claims do not have clear antecedent basis for removing engineered tissue from the membrane since tissue has not been previously required to be contained by the membrane. The Examiner suggested that the phrase "on the membrane" be inserted after the phrase "tissue" in the last line of claims 1 and 15. Applicants thank the Examiner for his helpful suggestion. Claim 1 and claim 15 have been amended as suggested. Applicants respectfully submit that the amendment to claim 1 and claim 15 provide antecedent basis to claim 2 and claim 16. Therefore, Applicants respectfully request the Examiner withdraw the rejection and allow the claims to issue.

Applicants believe they have addressed all of the Examiner's rejections under 35 U.S.C. § 112, second paragraph. Consequently, Applicants respectfully request the Examiner withdraw the 35 U.S.C. § 112, second paragraph rejections and allow the claims to issue.

b. 35 U.S.C. § 102

Claims 1-8 and 14-19 were rejected in the Office Action under 35 U.S.C. § 102(b) as being anticipated by Matsumoto *et al.* (International Conference, Bone Morphogenetic Proteins 2002, June 7-11, 2000). Claim 14 has been cancelled, making the rejection of this claim moot. Regarding the rejection of the remaining claims, Applicants respectfully traverse. In order to establish a prima facie case of anticipation, the reference must teach every element of the claim. MPEP § 2131 Applicants respectfully submit that the Examiner has failed to prove a prima facie case of anticipation because Matsumoto *et al.* fail to teach every element of the claimed invention as amended.

Specifically, Matsumoto *et al.* fail to teach where the intervertebral disc cells are transfected with exogenous nucleic acid, a limitation required by amended claim 1 and amended claim 15. Matsumoto *et al.* demonstrate that the intervertebral disc cells may be cultured in the presence of OP-1; however, there is no teaching or suggestion that the cells can also be

transfected with exogenous DNA. Because Matsumoto *et al.* do not teach or suggest every element of the claimed invention, the 35 U.S.C. § 102 rejection is improper and should be withdrawn. Applicants respectfully request that the Examiner allow the claims as amended to issue.

c. 35 U.S.C. § 103

i. Matsumoto *et al.* in view of Masuda *et al.*

In the Office Action, the Examiner rejected claims 1-18 and 14-19 under 35 U.S.C. § 103(a) as being unpatentable over Matsumoto *et al.* (International Conference, Bone Morphogenetic Proteins 2000, June 7-11, 2000) in view of Masuda *et al.* (U.S. Patent No. 6,197,061 or U.S. Patent No. 5,451,060). As stated in §2143 of the MPEP,

> [t]o establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

Applicants respectfully submit that a *prima facie* case of obviousness has not been established.

Claim 14 has been cancelled, rendering the rejection of the claim moot. Regarding independent claim 1 and independent claim 15, Matsumoto *et al.* and Masuda *et al.*, even when combined, fail to teach or suggest all of the limitations in the amended claims of the present invention. Particularly, neither reference teaches where the intervertebral disc cells are transfected with exogenous nucleic acid. Because the references do not teach or suggest all of the claim limitations, the Examiner has failed to establish a prima facie case of obviousness. As the Examiner has failed to establish a prima facie case of obviousness against independent claims 1 and 15, a prima facie case of obviousness must also fail against claims 2-8 and 16-19, which depend from claim 1 and claim 15. Thus, Applicants respectfully request the Examiner withdraw the 35 U.S.C. § 103 rejection and allow the claims as amended to issue.

ii. Masuda *et al.* in view of Hanley Jr. *et al.* or Matsumoto *et al.* and Chiba *et al.*

Claims 1-8 and 14-19 were rejected under 35 U.S.C. § 103 as obvious over Masuda *et al.* (U.S. Patent 6,197,061 or U.S. 6,451,060), Hanley Jr. *et al.* (U.S. 6,080,579) or Matsumoto *et al.* (International Conference, Bone Morphogenetic Proteins 2000, June 7-11, 2000) and if necessary in further view of Chiba *et al.* (Spine, Vol. 23, 1998) or Chiba *et al.* (Spine, Vol. 22, 1997). Applicants respectfully traverse.

Claim 14 has been cancelled, rendering the rejection of claim 14 moot. Concerning the other pending claims, three prongs must be satisfied by the cited references either alone or in combination for a prima facie case of obviousness to be established. One of the prongs that must be satisfied is that the references either alone or in combination must teach each claim limitation of the presently claimed invention. However, the references cited by the Examiner simply fail to teach or suggest that the intervertebral disc cells be transfected with exogenous nucleic acid, a claim limitation found in all of the currently pending claims. Thus, the Examiner has failed to prove a prima facie case of obviousness for claims 1-8 and 15-19.

In light of the Examiner's failure to establish a prima facie case of obviousness, Applicants respectfully request the Examiner withdraw the 35 U.S.C. § 103 rejection of claims 1-8 and 15-19 and allow the claims to issue.

CONCLUSION

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested. Examiner Naff is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

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FORMATION OF TRANSPLANTABLE DISC-SHAPED TISSUES BY NUCLEUS PULPOSUS AND ANNULUS FIBROSUS CELLS: BIOCHEMICAL AND BIOMECHANICAL PROPERTIES

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[INTRODUCTION] Degenerative intervertebral disc (IVD) disease is associated with a decrease in the contents of matrix proteoglycans (PGs) and collagens. Few attempts have been made to repair damaged IVD by transplanting a matrix-rich tissue formed *in vitro* into the defect. Recently, we have developed a novel two-step culture method using alginate-recovered-chondrocytes (ARC) for the production of cartilaginous tissue *in vitro*; this method alleviates the need for using exogenous matrices [1]. The purpose of this study was to test the possibility of combining these approaches to form *in vitro* a cohesive tissue for transplantation *in vivo*.

[MATERIALS AND METHODS]

Cell Isolation and ARC Culture Method Bovine IVD cells from the tails of 14-18 month old steer were isolated by sequential enzyme digestion [2]. The ARC method was then used as follows to form discs *in vitro*. Annulus fibrosus [AF] cells and nucleus pulposus [NP] cells were separately cultured in beads of 1.2% low viscosity alginate (Keltone LV, Kelco) at 4 million cells/ml using daily changes of DMEM/F12 medium containing 20% FBS + OP-1 (200 ng/ml), 25 µg/ml ascorbate and 10 µg/ml gentamicin. The cells with their cell-associated matrix were recovered by centrifugation of alginate beads solubilized in the presence of sodium citrate after 10 days of culture in alginate. The pelleted cells were resuspended in complete medium containing 20% FBS and 200 ng/ml of OP-1, seeded onto a tissue culture insert with a porous membrane (Costar, Transwell: 0.4 µm pore size, 10 mm diameter) and maintained in daily changes of the same medium for up to 4 weeks.

Characterization of Engineered Tissues *In Vitro* After 2 and 4 weeks, the *de novo* formed tissue was separated in each case from the porous membrane; the weights (dry and wet) were measured and the tissue was subjected to biochemical analyses. Each tissue was also examined histologically. The contents of sulfated PG and DNA were measured by the DMMB method and Hoechst 33258-dye method, respectively. The content of collagen was measured by reverse-phase high-performance liquid chromatography. Compressive and tensile testing were performed to determine the equilibrium compressive modulus, H_{A0}, the hydraulic permeability at 15% of strain, k_{p15}, and the peak tensile stress σ_{max}. The data were analyzed statistically using ANOVA.

[RESULTS] After 2 weeks, tissues engineered from NP and AF cells had a disk-like structure and were easy to separate from the membrane (**Figure 1**). The presence of OP-1, at a concentration of 200 ng/ml, stimulated the formation of cohesive discs. Interestingly, both the wet and dry weights and also the thickness of the NP discs were significantly higher than those of AF discs (**Table 1**). The water contents of NP discs were also significantly higher than those of AF discs (**Table 1**). Significant PG accumulation was observed in both NP and AF discs, but especially in the former ($p<0.01$) (**Figure 2A**). On the other hand, the collagen content of the AF discs was greater than that of NP discs ($p<0.01$) (**Figure 2B**). H_{A0} and σ_{max} varied significantly with cell type (each $p<0.01$) but not culture duration ($p=0.47$ and 0.17, respectively). H_{A0} and σ_{max} of AF tissue were significantly higher (+170% and +270%, respectively) than those of NP tissue (**Table 1**). k_{p15} was lower for AF discs than NP discs ($p<0.05$), and increased with culture duration ($p<0.05$), without an interactive effect ($p=0.75$).

[CONCLUSION] The results show that the recently described ARC method used to form cartilaginous tissue *in vitro* can also be used to form a disc-shaped tissue by IVD cells. Importantly, the collagen content was higher and the ratio of PG / collagen was lower in the AF than in the NP tissue, consistent with the observation that AF cells form a more fibrous tissue than NP cells *in vitro* and with the observed different mechanical properties of the engineered tissues using AF and NP cells. The results obtained thus far suggest that IVD tissues may be engineered *in vitro* using different cell sources (AF and NP) and that this process can be stimulated by growth factors such as OP-1. It remains to be determined if such tissues can be transplanted *in vivo* to repair IVD defects and/or degeneration.

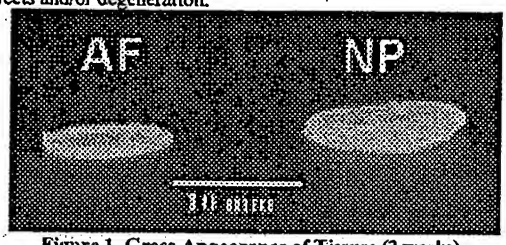

Figure 1. Gross Appearance of Tissues (2 weeks).

Table 1. Characterization of Engineered Tissues

	Culture Duration	AF disc	NP disc
Wet Weight (mg/tissue)	2 w	49.4 ± 1.7	132.7 ± 14.1 **
	4 w	159.4 ± 6.0	166.9 ± 5.2 *
Dry Weight (mg/tissue)	2 w	3.08 ± 0.09	7.21 ± 0.86 *
	4 w	5.52 ± 0.09	7.34 ± 0.25 ***
Water Content (%)	2 w	93.8 ± 0.3	95.8 ± 0.1 ***
	4 w	94.1 ± 0.1	95.6 ± 0.1 ***
Thickness (mm)	2 w	0.49 ± 0.13	1.37 ± 0.19 ***
	4 w	0.60 ± 0.17	1.11 ± 0.57 ***
H_{A0} (kPa)	2 and 4 w	2.44 ± 0.30	0.92 ± 0.16 **
σ_{max} (kPa)	2 and 4 w	250 ± 37	67 ± 13 ***
log (k_{p15})	2 w	-13.5 ± 0.2	-13.0 ± 0.3
	4 w	-13.0 ± 0.3	-12.4 ± 0.4

(*p<0.05, **p<0.01, ***p<0.005 versus AF)

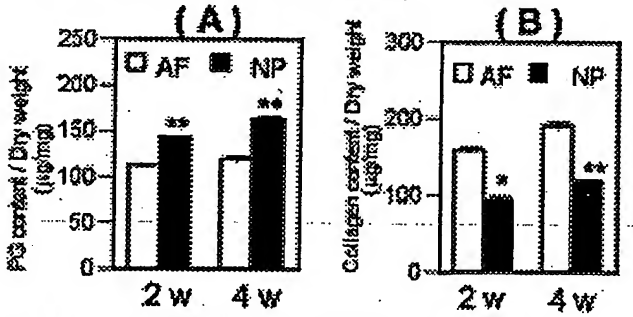

Figure 2. PG (A) and Collagen (B) Content of Engineered AF and NP Tissues (* p<0.05, **p<0.01 versus AF)

REFERENCES: 1. Masuda, K, et al. Trans ORS, 620, 2000, 2. Chiba, K et al. Spine, 22: 2285, 1997.

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